



**WHO International Standard
Calcitonin, Eel
NIBSC code: 88/556
Instructions for use
(Version 3.0, Dated 18/12/2007)**

1. INTENDED USE

The International Standard (IS) consists of a batch of ampoules which was established at the 40th Meeting of the WHO Expert Committee on Biological Standardization in 1989. Eel calcitonin has structural similarities with salmon calcitonin and, to date, research preparations have been evaluated with reference to the salmon calcitonin standard using the rat hypocalcaemia assay method. However, as salmon and eel calcitonins do have structural differences, which are likely to become evident if different *in vivo* or *in vitro* assay methods are used, a standard for eel calcitonin is required.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The preparation contains material of human origin, which has been tested and found negative for HBsAg, HIV antibody and HCV RNA by PCR.

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

Each ampoule of the IS contains 88 International Units (by definition).

4. CONTENTS

Each ampoule contains the residue after freeze-drying of 0.5ml of a solution which contained:-

Synthetic eel calcitonin	approximately 20µg
Albumin (human)	approximately 500µg
Mannitol	approximately 5mg

And pure dry nitrogen at slightly less than atmospheric pressure.

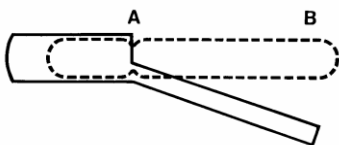
5. STORAGE

Unopened ampoules should be stored at -20°C.

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

For all practical purposes, each ampoule of the IS contains the same quantity of the substances listed. Dissolve the total contents of the ampoule in a known volume of a suitable solvent (buffer at pH 3.5) with carrier protein where extensive dilution is required. No attempt should be made to weigh out any portion of the freeze-dried material.

For economy of use, it is recommended that the solution be sub-divided into several small containers, frozen rapidly e.g. in dry ice and stored at -40°C or below. Careful evaluation will be needed to determine a feasible time of storage.

The ampoules do not contain bacteriostat and solutions of the IS should not be assumed to be sterile.

8. PREPARATION OF AMPOULES

Eel calcitonin manufactured by Bachem, USA, batch P1026 was generously donated to WHO for ampouling by Dr. F. Lattanzi and Dr R. Vanni, Sclavo SpA, Siena, Italy.

Analytical data provided by Sclavo stated that the peptide was >97% monocomponent and that the powder contained 5.56% moisture, 4.16% acetic acid and had a biological potency of 5061 units/mg in terms of the first International Reference Preparation of salmon calcitonin, ampoule code 72/158.

The filling conditions, and particularly the need to add albumin, were based on trial fills and stability evaluations carried out between 1982 and 1985. A research standard for eCT, ampoule code 84/587, had been prepared and included in the international collaborative study for the first International standard of the ASU¹⁻⁷ analogue of eCT (Unpublished report WHO/BS/85.1494).

The eel calcitonin was ampouled in July 1998. The peptide was dissolved in 0.001M acetic acid with 1% mannitol and 0.1% albumin and distributed into approximately 2,000 ampoules coded 88/556. Mean weight of filling solution was 0.50733, coefficient of variation 0.2316 (n=32).

After freeze-drying, ampoules were filled with pre dry nitrogen, sealed by glass fusion, tested for leaks and stored at -20°C in the dark. The ampouling was carried out according to procedures recommended by the WHO Expert Committee on Biological Standardisation (ECBS) (annex 4, 29th ECBS, 1978).

9. COLLABORATIVE STUDY

Seventeen laboratories in 10 countries took part in an international collaborative study. Each participant was asked to carry out at least two independent assays (i.e. using freshly reconstituted ampoules). As far as practicable each assay was to include the IRP sCT code 72/286 as calibrant, and the proposed International Standard eCT 88/556, each at 3 doses, with a minimum of 6 rats/dose in a single assay (or replicate wells for *in vitro* methods), so that assumptions of linearity and parallelism could be examined. Raw assay data from *in vivo* bioassays in 16 laboratories and *in vitro* bioassays in one laboratory were returned to NIBSC for centralized statistical analysis.

The statistical and computing methods used are based on those described in references 1-3. The proposed replacement International Standard for salmon calcitonin ampoule code 87/788 was included and calibrated in this study.

With the exception of one laboratory (for *in vitro* bioassays), all laboratories carried out the *in vivo* rat hypocalcaemia bioassay, usually according to the procedure described in the relevant pharmacopoeia although there were minor differences in the rat hypocalcaemia methodology reported by the 16 laboratories.

The *in vitro* bioassay carried out by one laboratory was based on the accumulation of cAMP by a breast cancer cell line known to have receptors for calcitonins (4).

The laboratory mean estimates for eCT, 88/556, in terms of the first IRP sCT, by *in vivo* bioassay were homogeneous with a weighted mean of 88 units per ampoule (95% confidence limits 85-91) if the mean estimate for one laboratory was excluded.



Estimates were also examined for any trends relating to *in vivo* bioassay details such as strain of rat, route of injection, etc. No consistent trends clearly attributable to methodology were detected. It is interesting to note that the *in vitro* bioassay appears to show a discrimination between eel and salmon calcitonins.

10. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

The qualitative and quantitative analysis of 20µg peptide in the presence of an excess of albumin (500µg) is not readily carried out by HPLC. Accelerated degradation samples of eCT were therefore examined in two independent *in vivo* bioassays by one laboratory. Resulting estimates of potency relative to samples of eCT stored continuously at -20°C were: for samples stored for 9 months at +20°C, 0.89 (95% confidence limits 0.75-1.06) and for samples stored for 9 months at +45°C (95% confidence limits 0.73-1.03). Estimates for samples stored at these two temperatures were similar with neither differing significantly from a relative potency of 1 so that no rate of degradation could be predicted. These data indicate that eCT 88/556 is sufficiently stable to serve as a standard.

11. REFERENCES

1. Finney, D.J. (1978). Statistical method in biological assay. 3rd edition. Charles Griffin & Co., London.
2. Gaines-Das, R.E. & Rice, L.R. (1985). SCAN, an exploratory program for preliminary analysis of bioassay and immunoassay data. Computer Methods and Programs in Biomedicine 21, 25-33.
3. Gaines-Das, R.E. & Tydeman, M.S. (1982). Iterative weighted regression analysis of logit responses. A computer program for analysis of bioassays and immunoassays. Computer Programs in Biomedicine, 15, 13-22.
4. Raue, F., Schneider, H-G., Zink, A. & Ziegler, R. (1987). Action of calcitonin gene-related peptide at the calcitonin receptor of the T47D cell line. Horm Metab Res, 19, 563-564

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr. F. Lattanzi and Dr R. Vanni, Sclavo SpA, Siena, Italy for donating the peptide, to the staff of the Standards Processing Division of NIBSC for ampouling and to all the participants in the study.

13. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
<http://www.who.int/biologicals/en/>
JCTLM Higher order reference materials:
<http://www.bipm.org/en/committees/jc/jctlm/>
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
<http://www.nibsc.org/products/ordering.aspx>
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

14. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

15. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

16. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties	
Physical appearance: Freeze dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Contains material of human origin	
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

17. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.



18. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 6mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biologicalstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.